

## Effect of salt stress on growth, electrolyte leakage, Na<sup>+</sup> and K<sup>+</sup> content in selected plant species

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**Abstract:** This study monitors the effect of salt stress induced by a NaCl solution (0 – deionized water, 50, 100, 200, 300 mmol/L) in lettuce (*Lactuca sativa* L. cv. Orion), New Zealand spinach (*Tetragonia tetragonoides* (Pall) Kuntze) and common purslane (*Portulaca oleracea* L. cv. Green Purslane) over the course of 50 days. The diverse reactions of these monitored species to salt stress are well apparent from the results. Lettuce proved as the most sensitive to salt stress, showing a significant reduction of dry weight, where even lower concentrations of salt affected membrane stability through increased electrolyte leakage value and an imbalance in the content of Na<sup>+</sup> and K<sup>+</sup>, observed in the form of lower ratios of K<sup>+</sup>/Na<sup>+</sup>. In case of *T. tetragonoides*, lower salt concentrations positively affected growth and this species appears to particularly accumulate sodium. In case of *P. oleracea* no significant reduction of dry weight took place with the increasing concentration of NaCl and a naturally high content of potassium contributed to maintaining a favourable ratio of K<sup>+</sup>/Na<sup>+</sup> even at higher salt concentrations, which is one of the prerequisites of salt-stress tolerance.

**Keywords:** salt tolerance; salinity; toxicity; stress response; ion imbalance

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants, because most are sensitive to salinity caused by high concentrations of salts in the soil, and the area of land affected by it gradually increases (Shrivastava and Kumar 2015). A concept of saline agriculture was proposed for rehabilitation of saline and degraded lands (Nikalje et al. 2017).

Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes (Akbarimoghaddam et al. 2011). Mahajan and Tuteja (2005) stated that salinity negatively influences germination and plant growth, as well as physiological processes (photosynthesis, respiration and transpiration), nutrient balance, membrane properties and cellular homeostasis, enzymatic and metabolic activities. Many authors, such as Sun et al. (2016) stated that the

salinity decreased photosynthetic carbon assimilation, stomatal conductance and lowered photosynthetic electron transport efficiency. Among the most destructive effects of salt stress is the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in plant tissues. The entry of both ions into the cells causes major ion imbalance and the excessive absorption may cause significant physiological failures (James et al. 2011). There are three adaptation physiological strategies to cope with excessive amounts of Na<sup>+</sup> and Cl<sup>-</sup>: (a) osmotic stress tolerance; (b) Na<sup>+</sup> and Cl<sup>-</sup> exclusion from leaves, and (c) tissue tolerance to Na<sup>+</sup> and Cl<sup>-</sup> accumulation (Munns and Tester 2008). Very important is regulation of transport and distribution of ions in different plant parts; Gu et al. (2016) reported that in cabbage seedlings an increase in Na<sup>+</sup> and Cl<sup>-</sup> concentration in roots, stems and leaves of cabbage seedlings was the main contributor to declining ratios

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of  $K^+/Na^+$ . Osmotic adjustment has an important role in salt adaptation mechanism (Flowers and Colmer 2008). For effective salt adaptation mechanism, efficient perception of stress signal and rapid activation of downstream processes are very important (Nikalje et al. 2017).

Plants are divided according to their sensitivity to salt-stress to glycophytes and halophytes. Glycophytes are not salt-tolerant (Zakharin and Panichkin 2009), where the majority of flora on this planet is composed of glycophytes. Carillo et al. (2011) stated that a plant might die should the concentration exceed 100–200 mmol NaCl. Dajic (2006) established that in cases of salt content in soil higher than 0.01%, glycophytes show slower growth. Halophyte plants are naturally able to grow in soil with higher concentrations of salt. They do not have problems with salinity exceeding 300–400 mmol NaCl (Cheeseman 2015). Most halophyte species are found in families Chenopodiaceae, Poaceae, Fabaceae and Asteraceae (Aslam et al. 2011). The potential applications of halophytes in phyto-remediation, desalination, secondary metabolite production, food and saline agriculture were discussed and the harvested halophyte can have industrial value and later on, rehabilitated soil can be utilized for agricultural purpose (Nikalje et al. 2017).

*Lactuca sativa* is widespread leaf vegetable with many varieties. Lettuce is categorized as being moderately salt tolerant. *Tetragonia tetragonoides* is a perennial plant used in salads, it is suitable for warmer climates and is salt tolerant. New Zealand spinach is reported to have medicinal uses (Ryuk et al. 2017). *Portulaca oleracea* is a drought and salt tolerant annual plant which contains high amounts of beneficial antioxidant vitamins and minerals (Uddin et al. 2012). The goal of our experiment was to evaluate the effects of various NaCl concentrations on growth, electrolyte leakage, as well as  $Na^+$  and  $K^+$  content in leaves of species with different sensitivity of salt stress.

## MATERIAL AND METHODS

**Plant material and experimental conditions.** The experiment focused on monitoring of the effects of salinity induced by NaCl on *Lactuca sativa* L. cv. Orion, *Tetragonia tetragonoides* (Pall) Kuntze and *Portulaca oleracea* L. cv. Green Purslane. The experiments took place in the greenhouse. They were conducted under semi-controlled conditions (natural light conditions, air temperature  $20 \pm 2/15 \pm 2^\circ\text{C}$  day/night, relative air humidity 65% min and 85% max).

The experimental plants were grown in containers with the volume of 5 dm<sup>3</sup> in the garden substrate (AGRO CS: pH 5.0–6.5, nutrient content 100 mg N/L, 44 mg P/L, 124 mg K/L). During the experiment, mineral nutrients were not added. Salinity was induced in the 16 BBCH phase (six fully developed leaves) in concentrations of: 0 (deionized water, control variant), 50, 100, 200 and 300 mmol/L NaCl. Watering took place over the course of 50 days in the amount of 50 mL of the solution every other day. Measurement of the monitored parameters occurred at the end of the experiment.

**Growth of plants.** The plants were harvested after fifty days of exposure. Dry weight (DW) of shoots (stem with leaves) was recorded together for five plants from salinity combination in three replications. The dry weight of shoots was determined after drying samples at 80°C until they reached a constant weight. The dry weight was then expressed per plant.

**Electrolyte leakage (EL).** Ten discs of fresh leaf (0.5 cm diameter) were cut from the fully expanded leaves (five plants per variant) and the samples were washed three times with deionized water to remove surface-adhered electrolytes. Leaf discs were placed in closed tubes containing 5 mL of deionized water and incubated at 10°C for 24 h. Subsequently, the initial electrical conductivity of the solution (EC1) was determined using a conductometer GRYF 158 (GRYF HB, Ltd., Czech Republic). The samples were then incubated in a water bath at 95°C for 20 min to release all electrolytes, cooled down to 25°C and their final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was calculated from  $EL = (EC1/EC2) \times 100$  (%).

**$Na^+$  and  $K^+$  content.** The samples were weighed on Teflon plates. Approximate weight ranged from 0.260 g to 0.290 g. Each sample was weighed in two replicates. The Teflon plates were then transferred into DAP-60S pressure mineralization tubes and 2 mL  $HNO_3$  (67%, Analytika, Prague, Czech Republic) and 3 mL of  $H_2O_2$  (min. 30%, Analytika, Prague, Czech Republic) was added. The contents were mixed and allowed to react for 90 min. The tubes were placed in the rotor of a Berghof MWS-3+ Speedwave microwave (Berghof Product + Instrument, Eningen, Germany). The time of the mineralization program was 60 min with a temperature range of 100–190°C.

The mineralizates were then transferred to 50 mL beakers and evaporated to a moist residue on a heating plate with a regulator at 150°C (Altec, Chotěboř, Czech Republic). After evaporating the liquid por-

tion of the samples, the beakers from the heating plate were removed and 1.5% HNO<sub>3</sub> was added to each. The contents were then quantitatively transferred into tubes and made up to a final volume of 25 mL with demineralized water. Analysis of their Na<sup>+</sup> and K<sup>+</sup> content was performed by a flame AAS method using Varian SpectrAA 110 (Varian, Mulgarve, Victoria, Australia) with SIPS at a suitable wavelength for a specific element –  $\lambda = 589.0$  nm (Na) and  $\lambda = 769.9$  nm (K).

**Statistical analysis.** A statistical evaluation of the experiment was made using the analysis of variance (ANOVA) and the values obtained were compared in further detail, using an *LSD* (least significant difference) test at the significance level  $P < 0.05$ . Statistical analyses were performed using Statistica 9.0 CZ for MS Windows software (Tulsa, USA).

## RESULTS AND DISCUSSION

**Plant growth.** The saline growth medium causes many adverse effects on plant growth, due to a low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances, or a combination of these factors Ashraf (2004). After 50 days of exposure, salt-stress affected the growth of the aboveground biomass differently in the monitored species.

In case of *L. sativa* dry weight significantly decreased along with increased salt concentration. Significant necrosis of leaf edges was observed from 100 mmol/L NaCl concentration (figure not shown), indicating lettuce sensitivity to salinity. Geilfus (2018) stated that the visual Cl<sup>-</sup> toxicity symptoms in the leaves start with chlorotic discolorations that may turn into necrotic lesions. At the concentration of 300 mmol/L NaCl, the dry weight decreased by 68% in comparison with the control group. The initial growth reduction was caused by the osmotic effect of salt outside the roots, and subsequent growth reduction was caused by the inability to prevent salt from reaching toxic levels in transpiring leaves.

Also Al-Maskri et al. (2010) consider lettuce sensitive and Di Mola et al. (2017) noting that the number of leaves, fresh and dry plant weight, as well as leaf area are significantly affected by salinity levels. Some varieties are considered moderately sensitive, for example, *L. sativa* var. *crispa* (Ünlükara et al. 2008). Also Bartha et al. (2015) states that there is a broad range of salt tolerance of different lettuce cultivars and, in fact, some degree of salinity may increase the marketable yield of this vegetable.

On the contrary, in case of *T. tetragonoides*, dry weight increased at the salinity level of 50 mmol/L NaCl by 31% in comparison with the control group. An evident decrease of dry weight in comparison with the control group was shown at concentrations of 200 and 300 mmol/L, by 17% and 39%, respectively. Similarly, Yousif et al. (2010) recorded an increased growth of *T. tetragonoides* at concentrations of 50, 100 and 200 mmol/L NaCl, which could be considered a characteristic of halophyte species. Kim et al. (2011) noted an increase in plant height, fresh and dry weight in *T. tetragonoides* with increasing saline irrigation.

In case of *P. oleracea*, salt stress evidently decreased dry weight to the level of 50 mmol/L NaCl by 32% in comparison with the control group (Figure 1). The differences in dry weight were no longer significant with increasing salt concentrations (100, 200 and 300 mmol/L NaCl). To the contrary, Kafi and Rahimi (2011) note reductions in root growth and shoot biomass accumulation at 120 and 240 mmol/L NaCl.

**Na<sup>+</sup> and K<sup>+</sup> content.** Na<sup>+</sup> content increased in all monitored species with increasing concentrations of NaCl in irrigation water (Figure 2). In *L. sativa*, the Na<sup>+</sup> content in leaves at the level of 300 mmol/L NaCl was 62.4 mg/g DW, which is five times more than the control group. In case of *P. oleracea*, Na<sup>+</sup> content increased 3.4 times (71.9 mg/g DW) and in *T. tetragonoides* 1.6 times (76.9 mg/g DW). Increased Na<sup>+</sup> content along with increasing salt concentrations were reported for lettuce (Ünlükara et al. 2008), New Zealand spinach Yousif et al. (2010) and purslane (Uddin et al. 2012). High Na<sup>+</sup> content inhibits the uptake of K<sup>+</sup> ions which is an essential element for growth and development (James et al. 2011). According to Tester and Danenport (2003) the key mechanism of salt tolerance is the ability of plants to regulate Na<sup>+</sup> uptake from soil. *T. tetragonoides* has shown the highest Na<sup>+</sup> content in all versions of salt stress, including the control group. Neves et al. (2008) stated that the *T. tetragonoides* has high salt removal potential, high biomass production potential, high content of minerals, etc.

Salinity caused by high concentrations of NaCl can reduce growth by the accumulation of high concentrations of both Na and Cl ions simultaneously, but the effects of the two ions may differ. High Cl<sup>-</sup> concentration reduces the photosynthesis and quantum yield due to chlorophyll degradation. High Na<sup>+</sup> interferes with K<sup>+</sup> and Ca<sup>2+</sup> nutrition and disturbs efficient stomatal regulation, which results in a depression of photosynthesis and growth (Tavakkoli et al. 2010).

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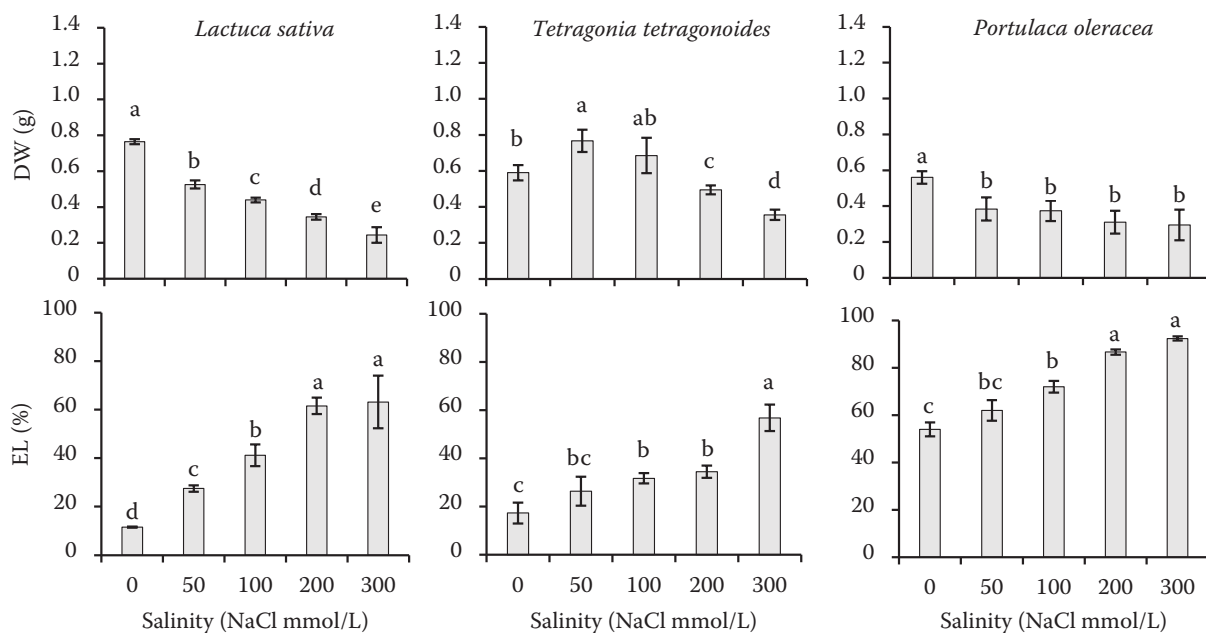


Figure 1. The dry weight (DW) of plants (shoots) and electrolyte leakage (EL) in relation to NaCl concentration. DW: one-way ANOVA, *LSD* (least significant difference) test; *Lactuca sativa*:  $F_{(4, 10)} = 195.43$ ,  $P = 0.00001$ ; *Tetragonia tetragonoides*:  $F_{(4, 10)} = 25.827$ ,  $P = 0.00003$ ; *Portulaca oleracea*:  $F_{(4, 10)} = 8.3165$ ,  $P = 0.00325$ . EL: one-way ANOVA, *LSD* test; *Lactuca sativa*:  $F_{(4, 6)} = 10.596$ ,  $P = 0.00001$ ; *Tetragonia tetragonoides*:  $F_{(4, 7)} = 11.611$ ,  $P = 0.00329$ ; *Portulaca oleracea*:  $F_{(4, 9)} = 29.249$ ,  $P = 0.00004$ . Values (means  $\pm$  standard deviation) sharing the same letters are not significantly different

$K^+$  content due to salt stress in comparison with the control group decreased in all species (Figure 2). However, in case of *L. sativa* and *T. tetragonoides*, the differences between the individual salt concentrations were statistically not significant. To the contrary, Bartha et al. (2015) reported that selected cultivars of lettuce have shown evident differences in potassium content between 50 and 100 mmol/L NaCl concentrations. Identically, Yousif et al. (2010) reported insignificant differences in potassium content in *T. tetragonoides* between 50, 100 and 200 mmol/L NaCl concentrations. In case of *P. oleracea*, potassium content gradually decreased with increasing concentrations of NaCl. Differences in potassium content were established among the monitored species. In case of the *P. oleracea* control group, potassium content was 113.4 mg/g DW, which is on average 2.3 more than in *L. sativa* and *T. tetragonoides*. Alam et al. (2014) also stated that potassium was present in the highest amount among all other minerals.

The  $K^+/Na^+$  ratio decreased in all species with increasing NaCl concentrations (Figure 3). Retention of a high  $K^+/Na^+$  ratio is defined as a determinative trait in salt tolerance (Fakhfeshani et al. 2015). The minimum value of  $K^+/Na^+$  is about one (Maathuis

and Amtmann 1999). *L. sativa* showed  $K^+/Na^+$  ratio lower than one in the 200 and 300 mmol/L NaCl concentrations. In case of *P. oleracea*, the ratio did not drop below this level in any salt stress concentration. In case of *T. tetragonoides* the  $K^+/Na^+$  ratio was generally low in all variants, including the control group, where the  $K^+/Na^+$  equalled one and further decreased with increased salt stress. These low values are caused by high ratios of  $Na^+/K^+$ , due to the high content of sodium in the tissues.

**Electrolyte leakage.** Plasma membranes are the primary site of ion-specific salt injury (Mansour and Salama 2004). Therefore, electrolyte leakage from plasma membranes is reported as one of the most important selection criterion for identification of salt-tolerant plants (Ashraf and Ali 2008). The EL value increased in all species with increasing salt concentrations (Figure 1). In case of *L. sativa*, evident significant differences were between the control group and the 50 mmol/L NaCl (27.5%). Similarly, Mahmoudi et al. (2011) observed EL indexes of the leaves and roots of lettuce progressively increased with increasing NaCl concentrations.

In case of *T. tetragonoides* and *P. oleracea*, significant increases of EL in comparison to the con-



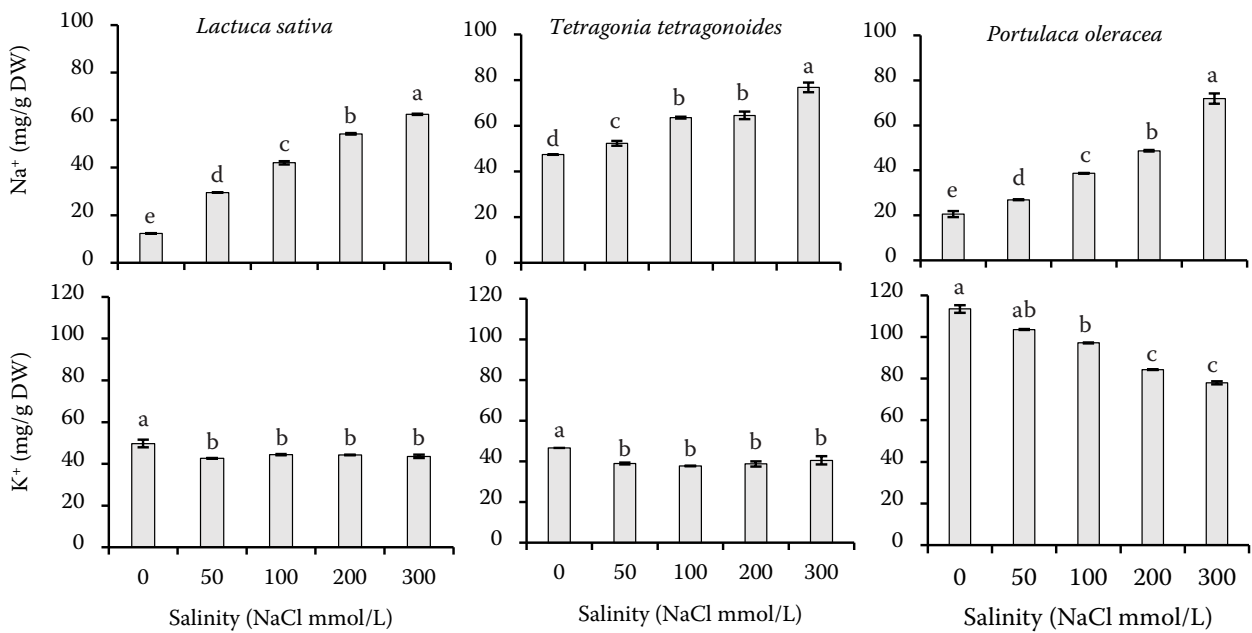


Figure 2. The content of Na<sup>+</sup> and K<sup>+</sup> in relation to NaCl concentration (means ± standard deviation; LSD (least significant difference) test,  $P < 0.05$ ). Na<sup>+</sup>: one-way ANOVA, LSD test, *Lactuca sativa*:  $F_{(4,5)} = 5997.8$ ,  $P = 0.00001$ ; *Tetragonia tetragonoides*:  $F_{(4,5)} = 152.84$ ,  $P = 0.00002$ ; *Portulaca oleracea*:  $F_{(4,5)} = 584.36$ ,  $P = 0.00001$ . K<sup>+</sup>: one-way ANOVA, LSD test, *Lactuca sativa*:  $F_{(4,5)} = 19.171$ ,  $P = 0.00310$ ; *Tetragonia tetragonoides*:  $F_{(4,5)} = 20.800$ ,  $P = 0.00257$ ; *Portulaca oleracea*:  $F_{(4,5)} = 23.988$ ,  $P = 0.00184$ . Values (means ± standard deviation) sharing the same letters are not significantly different; DW – dry weight

tol group took place from 100 mmol/L NaCl on (31.7% and 72.0%, respectively). The highest EL were established in *P. oleracea* on the levels of 200 and 300 mmol/L NaCl, 86.7% and 92.4%, respectively. In this case, high EL values may not be considered a sign of damaged plasma membranes due to stress, as also reported by Mansour and Salama (2004) but related to a naturally high content of potassium. Demidchik et al. (2014) also stated that electrolyte leakage is mainly related to the efflux of K<sup>+</sup>, which is abundant in plant cells.

The diverse reactions of the monitored species are well apparent from the results. Lettuce responded to salt stress with the highest sensitivity, which showed as a significant reduction of dry weight and even lower concentrations of salt affected membrane stability through increased EL value. An imbalance in ion content in the form of lower K<sup>+</sup>/Na<sup>+</sup> ratio was observed. *T. tetragonoides* and *P. oleracea* may be considered salt-tolerant species. In case of *T. tetragonoides*, lower concentrations of salt positively affected growth. This species, in particular, appears to accumulate sodium, which indicates a potential for further research, given the increasing amount of salt-stressed areas. In case of *P. oleracea*,

no significant reduction of dry weight was shown with increasing NaCl concentration and the naturally high potassium content contributes to maintaining a favourable K<sup>+</sup>/Na<sup>+</sup> ratio even in higher concentrations of salt stress, which is one of the prerequisites to salt-tolerance. Knowledge of response to salinity, adaptation and tolerance mechanisms of each species is a prerequisite for the selection of suitable salinity-tolerant species and genotypes. It is believed that the

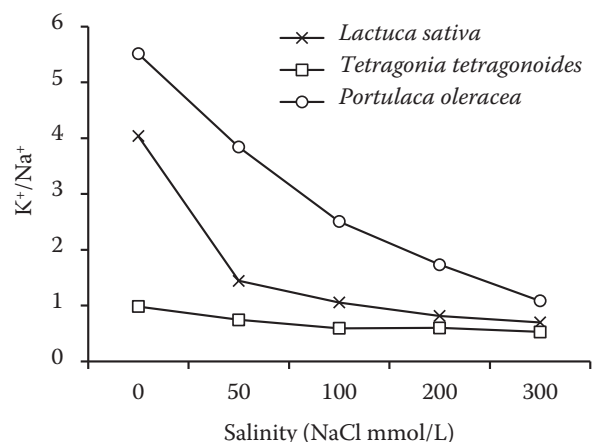


Figure 3. The K<sup>+</sup>/Na<sup>+</sup> ratio in relation to NaCl concentration

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Na<sup>+</sup> superaccumulator plant species (e.g. *T. tetragonoides*) appear to be usable in phytodesalination or co-cultivation with sensitive species; however, it requires further research.

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